

GAMETOGENESIS AND SPAWNING OF THE EUROPEAN OYSTER, *O. EDULIS*, IN WATERS OF MAINE

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In the late '40's a heavy mortality of an epizootic nature occurred among the populations of the soft clam, *Mya arenaria*, along the New England coast. The mortality was especially serious in the waters of Maine where, in many areas, these clams almost completely disappeared. Since *M. arenaria* was virtually the only commercial mollusk of that state, many shore communities were seriously affected economically. Realizing that economy established on a single shellfishery is insecure, we suggested that the local shellfisheries resources be supplemented by the introduction of another mollusk of commercial promise. We had in mind the European oyster, *Ostrea edulis*, which propagates at a somewhat lower temperature than our native oyster, *Crassostrea virginica*.

The European oysters were shipped to Milford from the Oosterschelde, Holland, in the fall of 1949. Large oysters arrived in good condition and, after resting in Milford Harbor, were planted in Boothbay Harbor and three other locations in Maine (Loosanoff, 1951, 1955). In Boothbay Harbor the oysters grew well and showed comparatively low mortality. Gonadal samples, taken from this group at regular intervals, provided the material for the histocytological studies on which the present paper is based. The tissue was preserved in Bouin's fluid, sectioned at 5 μ , and stained with Heidenhain's iron hematoxylin and eosin.

A description of the sexual phases of the European oyster is not the purpose of this article. This matter has been considered in a number of extremely informative publications, starting with Hoek (1883); Spärck (1925); and, especially, Orton (1927, 1933). Later it was reviewed by Coe (1936). Obviously, it is not necessary to go into the details of this complex problem again. Therefore, this article describes only two important aspects of the behavior of European oysters—gametogenesis and spawning under the new set of ecological conditions encountered in Boothbay Harbor.

It may be appropriate to mention here, chiefly as a reminder, Orton's discovery that the European oyster is protandric, with the sexual phases alternating regularly, in most individuals, after the initial male phase. Under favorable conditions each adult oyster completes one male and one female phase each year. Some oysters function as males early in the spawning season and later change to the female state. Others have the opposite sequence of phases. Because of this situation, individuals of both sexual types are found in the population during the entire spawning season (Cole, 1941).

Orton (1927) also suggested dividing the oysters into approximately eight arbitrary categories based upon the relative numbers of cells of different sexes in their gonads. These categories, which were established principally on studies

of gonadal smears, do not, however, appear to be precise enough if compared to results of similar studies in which regular histological preparations are used. The latter, based on a large number of sections, would show that different portions of a gonad of the same individual may often give an entirely different characterization of the sexual conditions of that individual. In other words, while some portions of the gonadal tissue of an individual may characterize it, according to Orton's standards, as a pure male, another and often closely adjacent portion of the gonad may display purely female conditions. Sometimes, in the same individual the adjacent follicles might contain cells of opposite sexes although the individual, as a whole, is, obviously, ambisexual.

Since virtually all of the Boothbay Harbor oysters studied were ambisexual to some extent, we hesitate to accept the classification of Orton, who assumed that there are pure male and pure female individuals which, presumably, contain exclusively male or female cells, respectively. Accordingly, in this study we shall designate all individuals as representing three chief categories, namely, (a) strongly ambisexual (Fig. 1); (b) predominantly male (Fig. 2); and (c) predominantly female (Fig. 3).

It is convenient for several reasons to begin the discussion of the seasonal sexual changes of the European oysters kept in Boothbay Harbor with the spring gametogenesis. Although in some instances it may begin in April, the population in general does not display early stages of gametogenesis until the latter part of May. Even at that time, approximately 10% of the oysters still possess typical winter follicles, which are small, contracted and surrounded by large masses of connective tissue (Fig. 4). At this time they contain only indifferent cells or gonidia, although phagocytic cells are often present in the lumina.

In oysters already undergoing spring gametogenesis, follicles in the same individuals are often in widely different stages of development. Although, in general, they are still small and surrounded by connective tissue, which occupies most of the space between digestive gland and outside membrane of the oyster body, a few sperm-balls are already present in males. In the majority of cases, however, only spermatids or spermatocytes have been formed so far. At this period, developing eggs are usually small, measuring under $50\ \mu$ (Fig. 5).

Two basic differences can be noticed between the general structure of the gonads of American and European oysters at this time and, also, later in the season. One is that the tissue layer for potential development of gonad material is significantly thicker in American than in European oysters of the same size. In the latter, the layer of gonadal tissue, *i.e.*, the area demarcated from the outside by the body wall and from the inside by the digestive gland, may very often consist of a single layer of follicles. Actual measurements show that while in American oysters 3" to 4" in size the thickness of gonadal layer may often be 3.5 to 4 mm., in European oysters of the same size it rarely exceeds 1.25 mm. Another basic difference is that while in American oysters the follicles are surrounded by a large number of leucocytes (lymphocytes) which probably act as carriers of nutritive material to the developing follicles (Loosanoff, 1942), cells of this type are entirely absent in *O. edulis* (Fig. 6).

By the middle of June the gonadal layer is still comparatively thin and only in exceptional cases do proliferating follicles extend all the way to the digestive gland.

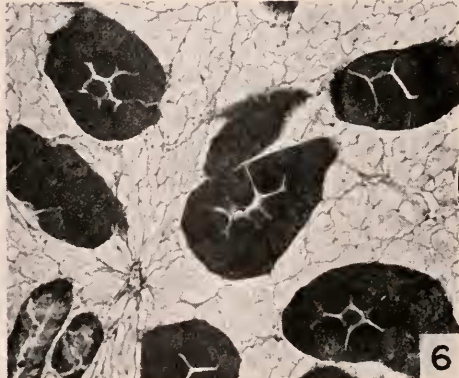
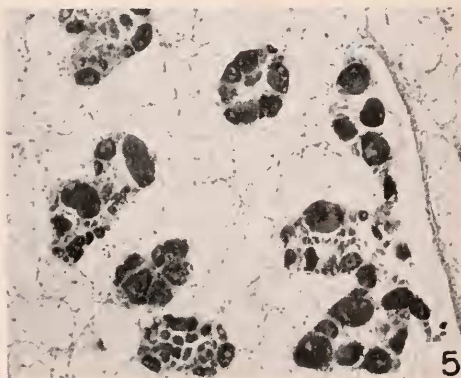
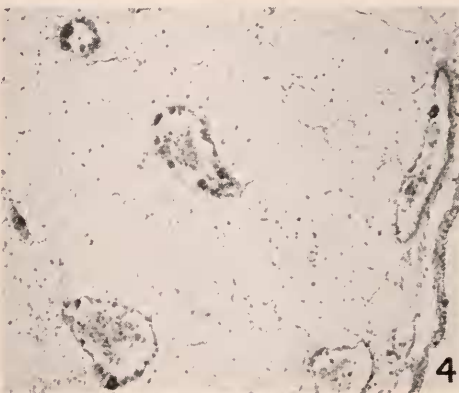
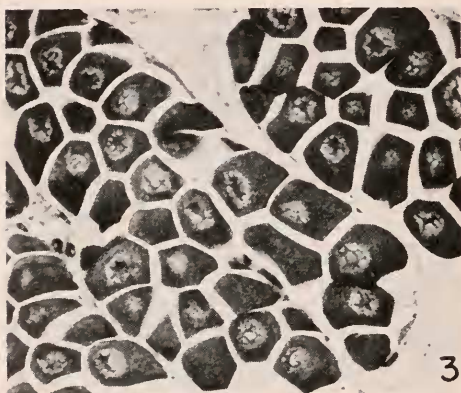
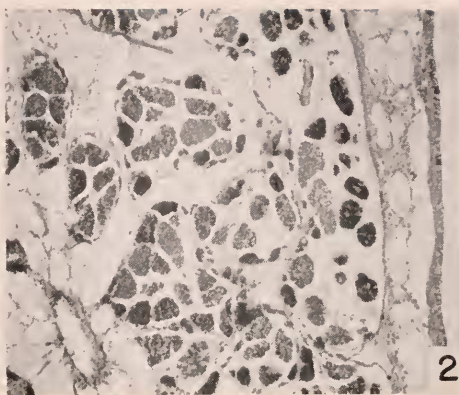
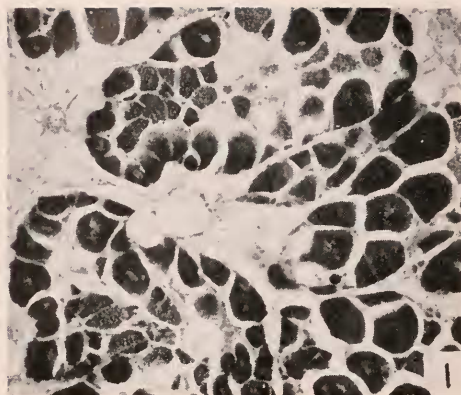


FIGURE 1. Ripe ambisexual gonad of *O. edulis*. Sperm-balls and groups of cells in earlier stages of spermatogenesis are in the center of the follicles. Note connections between blood vessels and apices of the follicles. $\times 80$.

FIGURE 2. Ripe predominantly male gonad of *O. edulis*. Many sperm-balls can be seen in genital canal. $\times 80$.

FIGURE 3. Ripe predominantly female gonad of *O. edulis*. $\times 80$.

FIGURE 4. Winter follicles of *O. edulis* confined between body wall and digestive gland

Nevertheless, more than half of the follicles with male cells contain what appear to be fully developed sperm-balls (Fig. 7). Spermatids become more numerous while primary and secondary spermatocytes begin to decrease in numbers. Eggs measuring between 75 and 100 μ are already found in some individuals, but there is no evidence of spawning.

At the end of June and during the first week of July sperm-balls can be found in the genital canals of some oysters, yet no mass spawning has occurred.

Spawning begins during the second or third week of July and continues until about the end of August (Fig. 8). However, during the first part of this period many oysters are still unripe and gonadal follicles occupy only about half the available space. In many males, even those which have discharged some sperm-balls, large groups of spermatids, and even spermatocytes, are still present.

Toward the end of July unripe oysters are still common but in the group representing only 5 to 10% of the population, spawning is already completed and resorption of gonads is beginning. In the latter individuals shrinking of follicles is in progress and invasion of connective tissue is rapidly taking place. Most of the individuals that have completed spawning are males but, strangely enough, regardless of early completion of the male phase, there is no cytological indication that a female phase will follow. It is probable, therefore, that in many instances, because of our short, cold summers these oysters can complete only one reproductive phase each season.

By the middle of August approximately 75% of the oysters are either partially (Fig. 9) or completely spawned. The majority of the completely spawned individuals, however, are predominantly males. As observed earlier in the summer, it is apparent that the male phase would not be followed by the completed female phase. Only in exceptional cases are small ovogonia or ovocytes seen developing along the walls of the follicles but, due to the lateness of the season, their development cannot progress very far before the onset of winter and relative inactivity.

After completion of the major spawning activities, the rest of the period of comparatively high temperature, confined chiefly to September (Fig. 15), is characterized by resorption of gonads and accumulation of glycogen. Resorption is brought about principally by phago-leucocytic cells which enter the follicles not through the follicular walls, as in American oysters (Loosanoff, 1942), but directly through the blood vessels (Figs. 10 and 11). Accumulation of glycogen is demonstrated by development of masses of connective tissue containing it, which rapidly fills almost the entire area between the digestive gland and the body wall of the oyster. The follicles, situated as islands within this tissue, are small, contracted and often filled with phagocytic cells (Fig. 12).

Strangely enough, simultaneously with completely spawned individuals there are still apparently normal oysters that have undischarged sex cells. These vary from those that retain only few cells in the follicles, or at least in genital canals, to those that carry virtually an undischarged supply of gametes. These individuals

and surrounded by large areas of connective tissue. $\times 80$.

FIGURE 5. Ambisexual gonad in early stages of spring gametogenesis. $\times 80$.

FIGURE 6. Predominantly female gonad during spring gametogenesis. Note absence of leucocytes (lymphocytes) outside of follicular wall, and connections between blood vessels and follicles. $\times 80$.

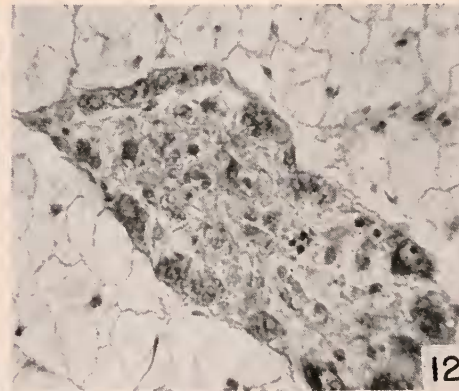
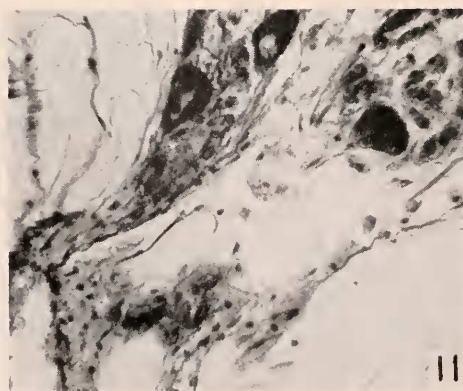
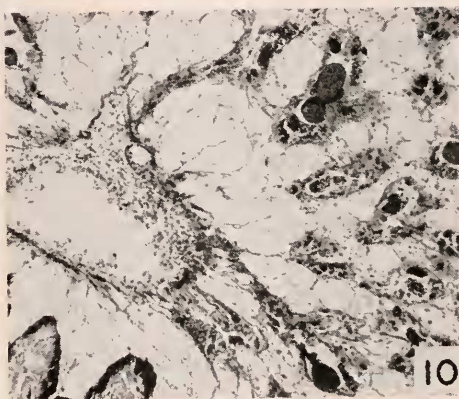
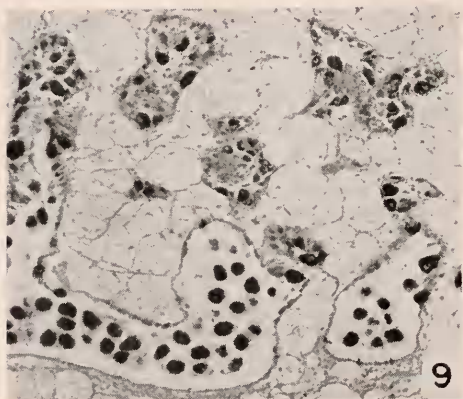
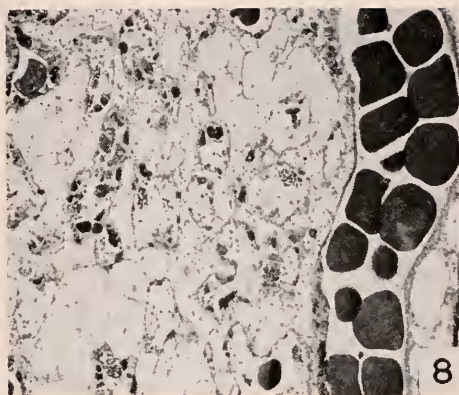
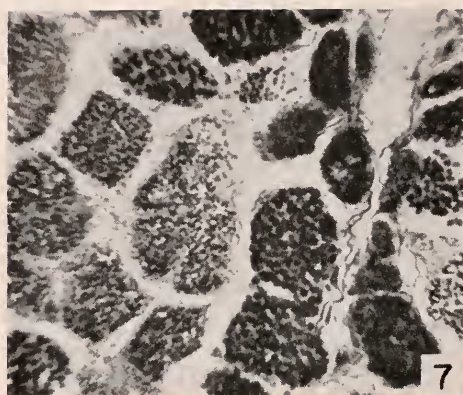


FIGURE 7. Portion of male follicles showing groups of cells in different stages of development. Sperm-balls, characterized by presence of tails of spermatozoa, are seen in the center. $\times 345$.

FIGURE 8. Ambisexual gonad during last stages of spawning. Remnants of male cells and some undischarged eggs can be seen in shrunken follicles, while a number of ripe eggs in the process of discharge are concentrated in the genital canal. $\times 80$.

may belong to all categories, including both strongly defined groups, *i.e.*, those predominantly males and those that are almost pure females.

A still more confusing aspect of the situation is that in some individuals with definitely male characteristics, spermatogenesis appears to be still in progress. Furthermore, in the same individuals, and sometimes even within the same microscopic field, follicles may be located side by side, some of which are completely discharged and already resorbed, others, only partly discharged, and the third group may contain male or female cells that seem to be undergoing healthy gametogenesis. Such situations can be encountered in samples collected by the end of October and, in a few instances, even later in the year.

According to Orton (1927) the proportion of females remaining unspent at the end of the summer in English waters varies from 0 to 5%. In our case, however, the proportion was significantly higher, in some samples exceeding 25%.

In discussing the fate of unspawned eggs, Orton (1927, p. 974) suggested that the oysters eventually dispose of these cells in two ways: some ova may be either retained in the gonad and become resorbed or "they may be included in egg-cysts and extruded in masses and excreted *en bloc* on to the internal face of the shell and covered over with nacreous or horny matter in the form of an excretion blister." While we agree with the first method suggested by Orton, we cannot accept his second suggestion which has not been supported by any reliable observations.

Striking differences in the condition of the gonad of the European oyster at the end of the spawning period in Boothbay Harbor probably suggest that we did not deal with a homogeneous population, but a group composed of representatives of physiologically-different races. Loosanoff and Engle (1942) and Loosanoff and Nomejko (1951) have demonstrated the existence of such physiologically-different races of the American oyster, *C. virginica*, along our Atlantic coast. These races require different minimum temperatures for development of gonads and inducement of spawning. In general, the breeding temperature requirements of the northern oyster are lower than those of the southern group. Korringa (1957) came to a similar conclusion regarding European oysters. He stated that the general population of that species is composed of several distinctly different physiological races which require different temperatures to carry on normal propagation activities.

Since French seed oysters are sometimes imported for cultivation in Holland, it is quite probable that within the sample sent to us there were individuals the genetic complex of which was such that their temperature requirements for reproduction were higher than those of the true northern oysters of Holland. As a result, because of the short, cold summers of Boothbay Harbor, these oysters were unable

FIGURE 9. Partly discharged predominantly male gonad. Groups of sperm-balls are seen in the genital canal, while many follicles are contracting and are invaded by phago-leucocytic cells. $\times 80$.

FIGURE 10. Resorption of gonad of *O. edulis* after spawning. Note shrinking follicles and connections between follicles and blood vessels. $\times 80$.

FIGURE 11. Migration of phago-leucocytic cells from blood vessels into apices of follicles. $\times 775$.

FIGURE 12. Follicles in last stages of resorption. Large group of phago-leucocytic cells occupying lumen. Young sex cells along follicular walls can be seen. $\times 345$.

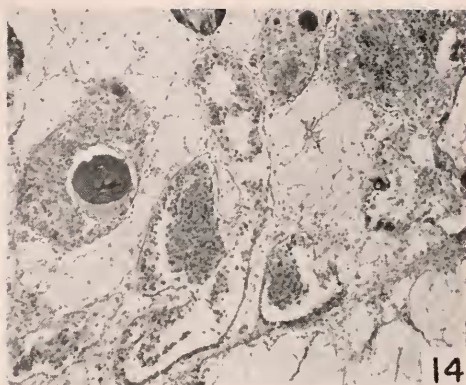
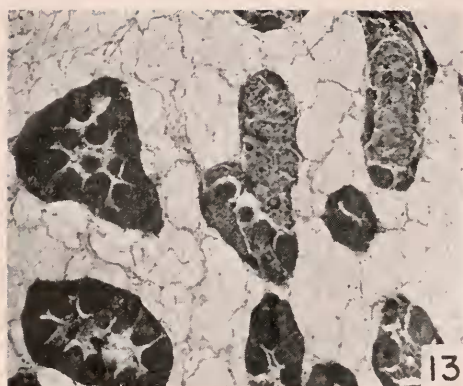


FIGURE 13. Cytolysis of predominantly female gonad that failed to ripen and be discharged. Note differences of degree of cytolysis of ovocytes in different follicles and also absence of phago-leucocytic cells outside follicular walls. $\times 80$.

FIGURE 14. Advanced stages of resorption of predominantly female gonad. Follicles are filled with large numbers of phago-leucocytic cells. $\times 80$.

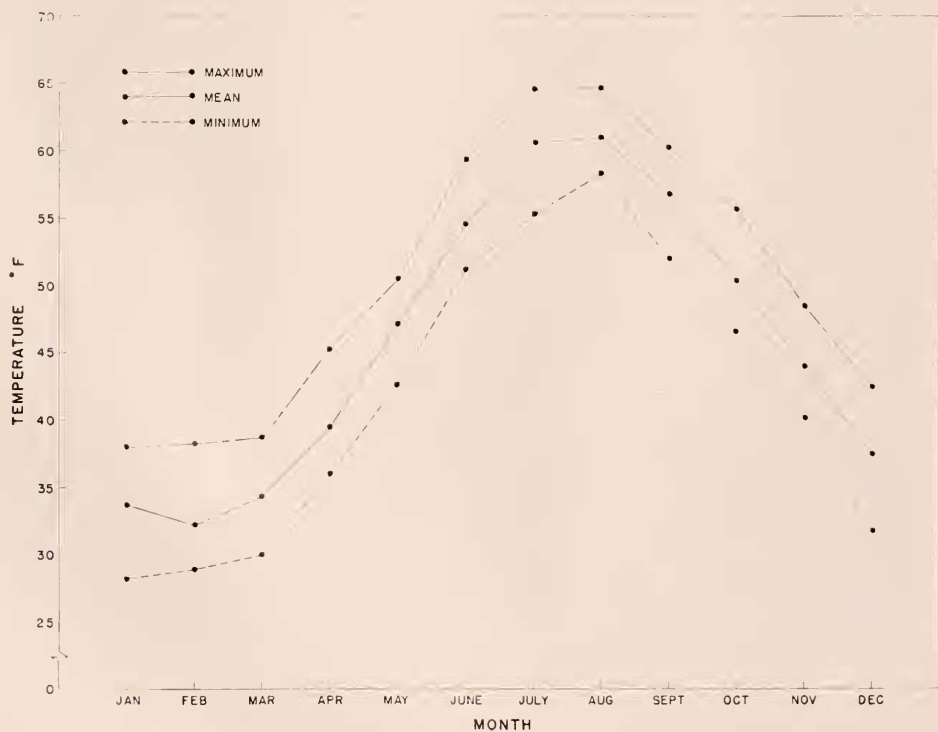


FIGURE 15. Mean, maximum and minimum monthly water temperatures at Boothbay Harbor, Maine during the period from 1906 to 1948.

to discharge their spawn. If the original shipment of European oysters, from which our samples for histological studies were collected, did include such races, it is quite probable that only the individuals carrying certain combinations of genes were able to propagate in Boothbay Harbor and are, therefore, responsible for the new population, which is now found in inshore areas of Maine (Welch, in press).

Studies of gonads collected late in November and December indicate that oysters can roughly be classified into two groups. In the first, resorption is almost or already completed. The follicles may be still full of phagocytes but, nevertheless, some young cells may be found along their walls. However, these cells are not differentiated.

The second group consists of individuals in which resorption of unspent sex cells is far from complete. Superficial examination may suggest that gonads are in the middle or at the beginning of the spawning period, but more detailed studies will show that cytolysis of undischarged cells, especially eggs, is in progress (Fig. 13). Thus, at this time of the year, as during other periods, there are pronounced individual differences between the condition of gonads of different oysters even if they belong, in general, to the same sex group.

Resorption of unspent cells continues through the winter. In some cases, it extends into April and perhaps even the first week of May, apparently depending upon individual conditions of the animals at the end of the preceding spawning season. However, at the same time and in the same oysters, in some of the follicles where resorption has already been completed, spring gametogenesis may be in progress. Consequently, two processes are going on simultaneously in the same individual—constructive gametogenesis and cytolysis. The latter is most active in female follicles where large groups of phagocytic cells attack remnants of the eggs (Fig. 14) which, in their appearance, greatly differ from each other, depending upon the stage of cytolysis.

As a rule, during early May no fully formed sperm-balls are found in the male follicles, but spermatids are occasionally seen. In female follicles normally developed oocytes are clearly distinguishable, and in well advanced females, follicles may be seen proliferating almost to the digestive gland. In this way the annual sexual cycle of *O. edulis* is completed and begins anew.

These studies were made possible through the cooperation of Messrs. John B. Glude and Walter R. Welch, of the U. S. Bureau of Commercial Fisheries, who were kind enough to take care of the European oysters kept in Boothbay Harbor and send me preserved samples of gonads. Special thanks are due to Messrs. Richard E. Reed, former Commissioner, Sea & Shore Fisheries, Maine, and Dana E. Wallace, who were extremely helpful in overcoming a number of difficulties in connection with the introduction of *O. edulis* into this country.

SUMMARY

1. Active spring gametogenesis begins in May.
2. General spawning begins approximately during the second or third week of July and continues until about the end of August.
3. Because of short, cold summers, usually only one sex phase is completed by an individual oyster.

4. Resorption of gonads is carried principally by phago-leucocytes which enter the follicles not through the follicular walls, as in American oysters, but directly through the blood vessels.

5. Resorption of gonads may continue throughout the winter and early spring.

6. Differences in conditions of gonads of *O. edulis* planted in Boothbay Harbor suggest that the original group was not composed of a homogeneous population, but consisted of different physiological races.

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